IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Appl. No.	:	**09/707,117**	Confirmation No. 8513
Applicants	:	**Jon A. Wolff, Vladimir Budker**	
Filed	:	**11/06/2000**	
Art Unit	:	**1632**	
Examiner	:	**Wilson, Michael C.**	
Docket No.	:	**Mirus.018.01**	

For: **Intravascular Delivery of Nucleic Acid**

Commissioner of Patents
PO Box 1450
Alexandria, VA 2231-1450

DECLARATION UNDER 37 C.F.R. §1.132

Dear Sir:

I, Dr. Sean D, Monoan, hereby declare as follows:

1. I have a Doctorate in Chemistry from the University of Wisconsin, Madison.
2. I am familiar with the above captioned application.
3. I am familiar with the development of transfection reagents for the delivery of nucleic acids into mammalian cells.
4. I am the author of the attached statement regarding the nature of polymers used for transfection..

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Dr. Sean Monahan 6-27-07

Dr. Sean Monahan Date

A significant number of multivalent cations with widely different molecular structures have been shown to induce condensation of DNA. Multivalent cations with a charge of three or higher have been shown to condense DNA. These include $Co(NH_3)_6^{3+}$,Fe^{3+} , and natural or synthetic polymers such as histone H1, and polylysine. Different polycations can generate different DNA/polymer complex formations and may require different positive charge to nucleic acid negative charge ratios to condense the nucleic acid. Also, nucleic acids of different size can affect polymer/nucleic acid complex formation. While condensation of DNA is attractive in forming transfection complexes, condensation alone is not sufficient. For example, polylysine is very effective at condensing DNA, but does not make an effective transfection reagent.

Microinjection is the mechanical injection of solution directly into a cell by means of a needle that pierces the cell membrane and optionally the nuclear membrane to deposit the solution directly into the cytoplasm of nucleus of a cell. Expression of DNA following microinjection of a DNA/polymer complex into cell, by itself, can not be considered to provide evidence that the polymer possess transfection activity or be able to delivery the DNA into the cell from outside the cell. To illustrate this point, microinjection of naked DNA into a cell results in very good expression of the DNA. However, application of naked DNA to the outside of cell will not result in any expression of the DNA in the cell.